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ORIGINAL ARTICLE

Brain regions implicated in inhibitory control and appetite regulation are activated in response to food portion size and energy density in children

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OBJECTIVE: Large portions of energy-dense foods drive energy intake but the brain mechanisms underlying this effect are not clear. Our main objective was to investigate brain function in response to food images varied by portion size (PS) and energy density (ED) in children using functional magnetic resonance imaging (fMRI).
METHODS AND DESIGN: Blood-oxygen-level-dependent (BOLD) fMRI was completed in 36 children (ages 7–10 years) after a 2-h fast while viewing food images at two levels of PS (Large PS, Small PS) and two levels of ED (High ED, Low ED). Children rated perceived fullness pre- and post-fMRI, as well as liking of images on visual analog scales post-fMRI. Anthropometrics were completed 4 weeks before the fMRI. Large PS vs Small PS and High ED vs Low ED were compared with region-of-interest analyses using Brain Voyager v 2.8.
RESULTS: Region-of-interest analyses revealed that activation in the right inferior frontal gyrus ($P = 0.03$) was greater for Large PS vs Small PS. Activation was reduced for High ED vs Low ED in the left hypothalamus ($P = 0.03$). Main effects were no longer significant after adjustment for pre-fMRI fullness and liking ratings (PS, $P = 0.92$; ED, $P = 0.58$).
CONCLUSION: This is the first fMRI study to report increased activation to large portions in a brain region that is involved in inhibitory control. These findings may contribute to understanding why some children overeat when presented with large portions of palatable food.

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INTRODUCTION

Childhood obesity is a global concern caused by a sustained positive energy balance beyond that required for growth.[1] Previous interventions designed to modify eating behaviors may lack long-term success because the underlying neurobiological mechanisms of overeating are poorly understood. One factor that drives overeating is portion size (PS) or the amount of food served.[2–4] Observational data suggest a positive relationship between PS of foods commonly eaten and body weight.[4,5] However, many children in the United States are not obese, despite living in an obesogenic food environment where large portions of palatable foods that are high in energy density (ED) are readily available.[6] This suggests that there may be individual differences in the susceptibility to large portions,[7] and investigating the underlying brain mechanisms involved in the response to food cues varying by PS and ED could help explain these differences.

Numerous laboratory studies have demonstrated that increasing food PS leads to increased energy intake in children,[2–4] which creates a risk for positive energy balance and weight gain over time. This is referred to as the PS effect.[8] Although the PS effect has been observed with foods that vary in ED, the greatest increases in energy intake are seen with large portions of high ED foods.[4,5] However, the mechanisms underlying the effects of PS and ED are not well understood.[8] Previous research indicates that visual cues of food PS (for example, amount of food available, positioning and shape) influence cognitive perceptions of how much energy is available and can facilitate changes in consumption,[9,10] food acceptance[11] and intake within a meal.[12,13] Further, food ED is known to have robust positive effects on energy intake, both independent of and in combination with large food PS.[14] Determining how the brain functions in response to visual cues of food PS and ED could clarify potential neurobiological underpinnings of the PS effect.

Advances in blood-oxygen-level-dependent (BOLD) functional magnetic resonance imaging (fMRI) have improved understanding of the neural correlates of eating behavior in youth. Similar to findings in adults,[15,16] studies in youth support the notion that high-calorie foods activate regions of the brain involved in reward processing, decision-making and inhibition.[17,18] The first published reports of fMRI food cue responding in healthy-weight adolescents (9–15 years) demonstrated significant activation in the limbic system (for example, hippocampus and parahippocampal gyrus) in response to food images, regardless of their energy content.[19,20] However, high-calorie vs low-calorie food images elicited greater activity in regions involved with conflict monitoring, object recognition and satiety processing.[20] Other studies have tested if the brain differentially responds to food cues varied by energy content[20–26] or palatability,[27–29] but results differ by weight status, sex and appetitive state.[21–23,25,26] In general, high-calorie foods evoke greater activation in brain regions implicated in energy homeostasis,[23,24] reward[25,30,31] and inhibitory

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control,[21,23,24,28] but additional research is needed to understand the food properties driving these effects.

An important limitation in previous neuroimaging paradigms is that stimuli were not well controlled for food ED or PS.[19,20,24,32,33] In addition, no previous fMRI studies have investigated the underlying brain activation associated with exposure to large portions of food. Furthermore, food images used in previous studies are often not customary for younger children (for example, a salmon filet), warranting the need for additional research using age-appropriate food stimuli. Moreover, most fMRI studies with food images have been conducted in adults[16] and children under the age of 10 years are underexplored. Developmental changes in brain anatomy and function occur between the ages of 4 and 18 years, especially in brain regions responsible for cognitive control and emotional drive.[34,35] These changes could make children particularly vulnerable to poor food intake decisions if the brain response to large portions overrides physiological needs. Further investigation is needed to better understand the neural mechanisms involved in response to PS and ED. These studies could lead to improved understanding of why some children are more susceptible to environmental food cues than others.

Our overall aim was to identify the brain regions engaged in response to visual food cues at two levels of PS (Large PS, Small PS) and two levels of ED (High ED, Low ED) in children (7–10 years). Based on the importance of visual–spatial cues on the perceptions of PS,[11,36] we hypothesized that brain regions involved in spatial integration (for example, hippocampus) would be responsive to Large vs Small PS. Based on evidence that amount consumed at a meal may be partly determined before a meal begins,[37] we anticipated that regions involved in executive function and decision-making (for example, dorsolateral prefrontal cortex) would be responsive to Large vs Small PS. In addition, according to studies that have found frontal inhibitory network engagement in response to food vs non-food cues,[15,26,32] we also hypothesized that inhibitory control regions (for example, inferior frontal gyrus (IFG)) would be activated in response to Large vs Small PS. Further, we anticipated that brain regions involved with reward processing, appetite regulation, taste and food salience (such as the orbitofrontal cortex) would be engaged in response to High ED vs Low ED. To our knowledge, this is the first study to characterize children's brain activation in response to variations in PS with food images. Characterizing the fMRI BOLD activation to food PS and ED may provide important insight into how the brain is influenced by these obesogenic environmental cues.

MATERIALS AND METHODS

Subjects

Thirty-eight children (97% Non-Hispanic White; 50% female) ranging in age from 7 to 10 years (mean 8.8 ± 1.2 years) participated in this study (Table 1). Parents with children were recruited by advertisements posted in the local community. Inclusion criteria were right-handedness (that is, to reduce variance in hemisphere-specific responding owing to handedness), reading at or above grade level, English as a native language and free of metal (for example, braces) to avoid effects on scan quality. Sixty-one families were screened by phone and 19 were excluded at screening based on criteria as follows for: medical/psychological disorders contraindicative of fMRI (for example, attention deficit hyperactivity disorder; $n=6$), left-handedness ($n=2$), food allergies ($n=2$), permanent metal dental work ($n=2$), and medication usage that may affect brain activity ($n=2$). Participants were enrolled in the 5-visit study ($n=42$) and completed an fMRI scan ($n=38$) on the fifth visit. Participants who did not complete the fMRI ($n=4$) moved out of state ($n=2$), acquired dental work during the study ($n=1$) or refused to be scanned ($n=1$). Because of excessive motion, two participants were removed from analyses. This resulted in the final sample of 36 children. Parental consent and child assent were obtained on the first visit. Participants were financially compensated after each visit. The Pennsylvania State University Institutional Review Board approved this study.

Table 1. Participant characteristics

	n	%
Sex		
Male	18	50
Female	18	50
Weight status		
Non-overweight	34	94
Overweight	2	6
Race		
Caucasian	33	92
Other	3	8
	Mean	*s.d.*
Age (years)	8.9	1.2
Percentage of body fat	16.4	6.5
BMI *z*-score	−0.2	0.8
Fullness (mm)		
Prescan	40.8	39.4
Postscan	36.3	39
Postscan liking of food images (mm)		
Large PS	101.3	23.2
Small PS	100.7	23.8
High ED[a]	112.7	24.7
Low ED[b]	89.3[c]	26.4
Postscan wanting of food images (mm)		
Large PS	96.0	27.7
Small PS	94.9	27.9
High ED	106.3	30.4
Low ED	84.6[a]	28.9

Abbreviations: BMI, body mass index; ED, energy density; PS, portion size. [a]ED cutoff for High >1.5 kcal g^{-1}. [b]ED cutoff for Low <1.5 kcal g^{-1}. [c]Significantly different vs rating for High ED ($P<0.001$).

Baseline anthropometrics

Researchers measured height, weight and body fat percentage of children in light clothing (shoes and coats removed) using a stadiometer (Seca model 202, Seca, Chino, CA, USA), standard scale (Detecto model 437, Detecto, Webb City, MO, USA) and bioelectric impedance analysis (Tanita model BF-350, Tanita, Arlington Heights, IL, USA). Body mass index (BMI) and additional weight status markers (BMI *z*-score, BMI Percentile) were calculated using measured height and weight based on the Centers for Disease Control and Prevention growth charts as the weight-to-height ratio for age and sex.[38]

Study design and summary of test sessions

Children participated in five laboratory visits to complete a variety of measures, including: anthropometrics, test meals, eating behavior questionnaires, fitness assessments, and the fMRI scan. Each visit was scheduled approximately 1 week apart and conducted at an eating behavior laboratory located in a university campus. To accommodate school schedules and extracurricular activities, test meals were conducted during typical lunch (1100–1300 hours) or dinner hours (1600–1800 hours). Participant testing times were kept consistent within child and balanced across children. Participants were instructed to refrain from eating for 2 h prior to each scheduled visit to maintain a consistent neutral appetitive state, defined by rated fullness level between 25% and 75% of a pictorial visual analog scale. Parents and children completed self-report and interview-based questionnaires (that is, demographics, measures of children's feeding/eating behavior) on the first visit. Test meals to assess PS response in children were consumed on visits 1–4 (data to be reported elsewhere).[39] Mock fMRI training sessions were completed with children after visits 3 and 4 to increase their exposure to the scanning environment. Children completed an fMRI on the fifth visit and were scanned in a neutral appetitive state to avoid potential increases in brain response to all food

cues (regardless of PS) if they were tested in a fasted state.[30,32] Data from visit 1 (that is, demographics and anthropometrics) and visit 5 (fMRI scan) are reported in this paper.

fMRI training

Training was conducted in a mock scanner (that is, contains no magnet but looks like the actual scanner) on two separate sessions after completion of the test meals. This fMRI training protocol was developed for the present study and has been detailed previously.[8] The first training session consisted of an introduction, where researchers observed the participants' comfort level by allowing the child to lead exploration of the mock scanner.

On the second session, children were positioned on the mock scanner bed, affixed with headphones and aligned with the mock head coil. Children were provided with emergency buttons to operate the mock scanner bed, as well as cushioning to reduce movement. Researchers provided instructions on the importance of lying still using relevant analogies, such as staying still as a statue, and participants practiced answering questions by speaking 'yes' or 'no' without head movement. Once comfortable, children viewed images that were not part of the fMRI paradigm (for example, animals) and listened to sounds they would hear in the actual scanning environment. If children had excessive movement during this procedure, they were asked to attend an additional training session on a separate day to repeat this protocol. One participant completed this additional session.

fMRI stimuli

Food images shown in the fMRI were created by using the Continuing Survey of Food Intakes by Individuals to identify the foods commonly eaten by children this age, as well as estimates of PS per eating occasion.[40] Thirty foods with ED $>$ 1.5 kcal g^{-1} and 30 foods with ED $<$ 1.5 kcal g^{-1} were selected (see Supplementary Table S1). We selected a moderate ED cutoff of 1.5 kcal g^{-1} (for example, macaroni and cheese) to control for large differences in palatability between the food groups. When possible, low and high ED versions of the same food were selected. For example, chicken nuggets (ED 2.4 kcal g^{-1}) were in the High ED category and grilled chicken strips (ED 1.3 kcal g^{-1}) were in the Low ED category. PSs for the food stimuli were determined by using amounts referenced in the Continuing Survey of Food Intakes by Individuals. For each selected low or high ED food, the Small PS condition was photographed at the 10th percentile of the amount commonly consumed, for an average of 18 kcal for Low ED foods and 74 kcal for High ED foods. The Large PS condition was photographed at the 90th percentile and averaged 89 kcal for Low ED and 370 kcal for High ED. Foods were photographed using a high-resolution camera (Canon PowerShot SX260 HS, Canon U.S.A., Inc., Melville, NY, USA) at a predetermined height from the ground to the camera lens (37.5") and depth from the plate of food to camera tripod (12.5"), providing an angle (52.8°) from which a typical child would view if seated at a dining table. Foods typically consumed in bowls (for example, yogurt) were photographed in white bowls (18 oz) while foods commonly eaten from plates (for example, meat) were photographed on white plates (10 ¼") (Corelle Livingware 'Winter Frost White' dinnerware, World Kitchen, Corning, NY, USA). All foods were photographed on dishware and were pasted onto a standardized background image of a blue linen tablecloth for optimal contrast.

Two control conditions were developed for this paradigm (Figure 1). Furniture images were chosen to control for object recognition and general interest (that is, objects that are not appetizing). Scrambled images were created to control for color and other low-level visual features and were not recognizable as either objects or foods. The Scrambled condition images were a subset of images from the other stimuli conditions that were pixelated and scrambled in Matlab v. 8.0 (Mathworks, Natick, MA, USA).

Image manipulation software (GIMP v. 2.8, a free and open source image editor at: https://www.gimp.org/) was used to manually adjust inconsistencies in color, size and depth perception of foods. Low-level visual differences in physical attributes of stimuli are important to consider in food cue responding.[41] Therefore, visual features of all stimuli were extracted from images, including stimuli size, brightness (that is, grayscale) and integrated density (that is, intensity), using Adobe Photoshop CC 2014.2.2. In general, each of the four food stimuli conditions had similar brightness and intensity compared with each other and compared with the Scrambled condition. Because differences in brightness were identified for Furniture stimuli relative to all other stimuli (data not shown), the Scrambled images were chosen to represent a 'baseline' control.

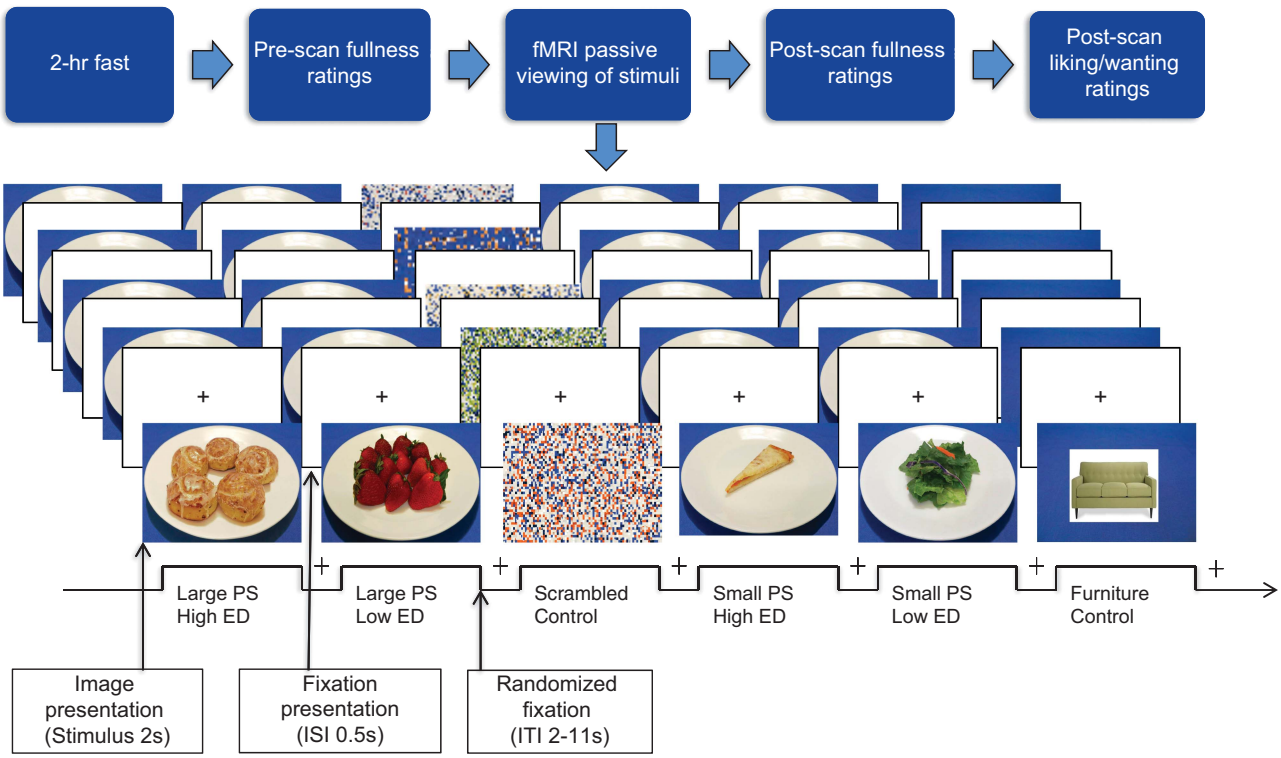

Figure 1. The study procedure involved participants arriving after a 2-h fast for the fMRI scan. Level of fullness was collected by visual analog scale before and after the scan. Liking and wanting ratings were completed immediately following the scan. The full fMRI battery included one anatomical scan (not depicted) and six functional runs. An example of one functional run with each condition is shown. Images were presented for 2 s each with a fixation in between stimuli of 0.5 s and a randomized fixation of 2–11 s between runs.

Table 2. Coordinates tested for main effects of portion size and energy density

Region of interest		*Talairach coordinates*		
		x	y	z
Amygdala[a]	R	22	−10	−10
	L	−22	−10	−10
Striatum (ventral)[a]	R	18	20	−6
	R	−18	20	−6
Insula[a]	R	36	−6	−12
	L	−36	−6	−12
Orbitofrontal cortex[b]	R	32	29	−3
	L	−32	29	−3
Orbitofrontal cortex[a]	R	36	28	−10
	L	−36	28	−10
Ventral tegmental area[c]	R	3	−17	−4
	L	−3	−17	−4
Ventromedial prefrontal cortex[d]	R	9	45	2
	L	−9	45	2
Ventromedial prefrontal cortex[d]	R	6	36	−14
	L	−6	36	−14
Red nucleus[e]	—	0	−18	−8
	—	0	−18	−12
Hypothalamus[f]	R	3	−1	−4
	L	−3	−1	−4
Inferior frontal gyrus[g]	R	50	4	16
	L	−50	4	16
Dorsolateral prefrontal cortex[g]	R	29	29	36
	L	−29	29	36
Dorsomedial prefrontal cortex[g]	R	5	51	24
	L	−5	51	24
Parahippocampal gyrus[g]	R	21	−48	2
	L	−21	−48	2
Cingulate (posterior)[b]	R	18	−46	0
	L	−18	−46	0
Fusiform gyrus[h]	R	28	−48	−12
	L	−28	−48	−12
Fusiform gyrus[h]	R	34	−62	−6
	L	−34	−62	−6
Hippocampus[h]	R	28	−24	−6
	L	−28	−24	−6

Abbreviations: L, left hemisphere; R, right hemisphere. [a]Schur *et al.*[26] [b]Dimitropoulos *et al.*[22] [c]Stoeckel *et al.*[25] [d]Hare *et al.*[43] [e]Tomasi and Volkow.[45] [f]Brett *et al.*[44] [g]Brooks *et al.*[15] [h]Killgore and Todd.[20]

fMRI design

Images were presented in a block design with six conditions of food and control stimuli. To determine the main effects of PS and ED, a 2 × 2 design (PS × ED) was used to create four conditions of food stimuli: Large PS High ED, Small PS High ED, Large PS Low ED, and Small PS Low ED. Two conditions of control stimuli are shown (Furniture, Scrambled).

The fMRI battery included six functional runs to obtain functional activity of the brain in response to food and control stimuli and one structural scan to determine brain anatomy. Within each functional run, blocks were presented in a pseudo-randomized order so that the child would not see >2 food blocks in a row before seeing a control block. Each block of stimuli was a set of five images that were chosen to maintain consistency of food type within blocks (Supplementary Table S1). Each image was presented only once throughout the entire fMRI battery. Figure 1 depicts an example of one functional run. Stimuli were presented for 2 s and with a 0.5-s fixation period between stimuli (that is, interstimulus interval) and 2–11-s jittered fixation period between blocks (that is, intertrial interval).

fMRI data acquisition

Scans were performed on a Siemens 3.0-Tesla MRI scanner (Siemens Medical Solutions, Erlangen, Germany) with a standard (12-channel) coil. Pillows, padding and headphones restricted head motion while adding comfort to participants. Structural scans were collected using a T1-weighted sequence (MP-RAGE) TR/TE = 1650/2.03 ms, flip angle = 9°, FOV = 256 mm, slice thickness = 1 mm, sagittal plane, voxel size 1 × 1 × 1 mm^3. Each structural scan lasted approximately 4 min. The functional scans used a T2-weighted gradient single-shot echo planar imaging sequence (TE = 25 ms, TR = 2000 ms, flip angle = 90°, matrix 64 × 64) with an in-plane resolution of 3 × 3 mm^2 (FOV = 220 mm) to acquire 33, 3 mm (interleaved) slices along the AC–PC plane. In-scan prospective movement correction was used.[42] Each functional run took approximately 3.5 min. Researchers checked participant comfort and alertness verbally between structural and functional runs. The fMRI battery was designed for completion in 25 min but varied from 21 to 35 min depending on scan performance and comfort.

Defining regions of interest (ROIs)

Table 2 lists the coordinates of ROIs tested specifically for main effects of PS and ED. Brain ROIs were predetermined and previously reported in food-related fMRI literature.[15,20,22,26,32,43] Specific coordinates of the predetermined ROIs were selected[15,20,22,25,26,43–45] and verified using Talairach Client (v. 2.4.3, a free java client/web applet at http://www.talairach.org/manual.html), software that provides the nearest gray matter to the inputted coordinates. Coordinates previously reported in Montreal Neurological Institute space[25,43] were converted to Talairach space using MNI2Tal (a free java applet available at http://bioimagesuite.yale.edu/mni2tal/; BioImage Suite 2.0, New Haven, CT, USA). Brain structures were included that have been considered part of the 'appetitive network',[46] known to be involved in modulating pleasure and motivation (limbic system), spatial integration (temporal lobe areas), reward processing (mesolimbic and somatosensory circuitry) and cognitive control (prefrontal areas). Brain regions were defined by drawing a 5-mm sphere in BrainVoyager QX software, v. 2.8.2 (Brain Innovation, Maastricht, The Netherlands) around the chosen coordinates for ROIs in both hemispheres.

Ratings for fullness, liking and wanting of test stimuli

Children rated perceived prescan and postscan fullness levels with a pictorial, 150-mm visual analog scale.[47] Immediately following the scan, children rated liking and wanting of the food and furniture images presented in a pre-established order of blocks that were pseudo-randomized so that the same foods were not presented in contiguous blocks. Children were asked 'How much do you like this food/item?' and 'How much do you want this food/item?' for images seen inside the scanner. They responded by pointing to the appropriate spot on a computerized 150-mm analog scale that was operated by the researcher. For the behavioral analyses, mean liking and wanting ratings for the images were calculated across conditions (for example, liking of Large PS foods; wanting of Large PS foods).

Data analysis

Behavioral data. Descriptive data were analyzed in SPSS 22.0 (IBM Corp., Chicago, IL, USA) and reported as means ± s.d. Postscan mean liking and mean wanting of food images by condition, by PS (collapsed across ED) and by ED (collapsed across PS) were computed using SPSS. Differences in prescan and postscan measures for fullness and liking were analyzed using paired *t*-tests. A *P*-value cutoff of 0.05 was used to assign significance.

fMRI data. All fMRI data preprocessing was completed in Brain Voyager as follows. Spatial normalization was conducted by manually converting anatomical data to Talairach[48] space using the AC–PC landmark and fitting six parameters (anterior, posterior, inferior, superior, left, right) on each subject's respective structural scan. Converting children's anatomical data to Talairach space allowed for normalization to a common stereotaxic space and comparison to brain regions implicated in the adult literature. Although some researchers suggest the use of child-specific templates,[49] others have argued that for children aged >6 years, differences in brain anatomy from adult samples are minimal and below the resolution of the scanner.[50] Functional data were preprocessed with 3D motion correction using six vectors (three translations and three rotations) and temporal filtering (high-pass filtering using a GLM-Fourier basis set with six cycles per time course) but were not smoothed. The first two functional volumes were discarded for all participants. Functional runs with excess motion (cutoff: 3 mm or 3° in any direction) were excluded from analyses. Anatomical data were precisely aligned and co-registered to preprocessed functional data. Only subjects who had one or more successful functional runs were allowed in the analyses. These criteria excluded 35 out of a total of 228 potential functional runs from 38 participants. Two participants had

no successful runs and were excluded. The final sample of $n = 36$ for analyses had a range of 3–6 runs and averaged 5.36 successful runs out of 6 possible.

Variables representing experimental conditions of interest (for example, Large PS High ED; Small PS High ED; Large PS Low ED; Small PS Low ED) were modeled with a boxcar that was convolved with a hemodynamic response filter. Volume time-course files for each participant for each successful run were entered into a general linear model using random effects in Brain Voyager. The ROI approach was chosen to determine the main effects of PS and ED within *a priori* selected ROIs (Table 2). In this approach, repeated measures two-factor analyses of variance (ANOVAs) were performed in Brain Voyager within the 36 ROIs to determine the main effects for PS by comparing the mean parameter estimates (β) of BOLD activation within each ROI to Large PS food images (Large PS High ED +Large PS Low ED) against the mean parameter estimates (β) of BOLD activation to Small PS food images (Small PS High ED+Small PS Low ED). Similarly, the main effects of ED were tested in Brain Voyager by comparing the mean parameter estimate (β) of BOLD activation within each ROI to High ED foods (Large PS High ED+Small PS High ED) against the mean parameter estimate (β) of BOLD activation within each ROI to Low ED foods (Large PS Low ED+Small PS Low ED). To confirm the analyses, β coefficients of BOLD activation to all conditions in the 36 ROIs were extracted from Brain Voyager, ANOVAs were rerun in SPSS and the results matched those from Brain Voyager. After the *P*-values were obtained for each of the 36 ROIs tested with ANOVA, these *P*-values were input into Benjamini–Hochberg correction[51] to correct for multiple testing. This approach to false discovery rate (FDR) correction is widely used[33] and was run to control for the expected proportion of falsely rejected hypotheses. This applied a corrected significance level $q = 0.05$ to the calculated *P*-values for 36 ROIs for PS and ED separately.

In the ROIs that survived correction for multiple comparisons (Table 3), analyses of covariance were performed. Selected variables likely to be related to the main effects of PS and ED (for example, prescan fullness[23,30] and liking of food images[28,52]) were input into repeated-measures analysis of covariance models in SPSS to determine whether they significantly influenced the relationship between condition and brain response. Because variance in BMI *z*-score and body fat percentage was low and did not significantly affect our models, neither was included as a covariate. Although the main objective of the current study was to determine the brain response to food-based comparisons varying by PS and ED, the response to food stimuli compared with non-food stimuli was assessed with paired *t*-tests. To provide additional perspective on how food stimuli activated brain regions relative to control images (Scrambled), the results are presented in Supplementary Table S2. We also included Supplementary Table S3, which presents results of the two-factor ANOVAs for PS and ED to show how the food-based comparisons activated all ROIs tested.

RESULTS

Descriptive statistics

Participant characteristics are presented in Table 1. As shown, there was an even split of boys and girls (mean age 8.9 ± 1.2 years). The majority of children were of healthy weight, with mean BMI *z*-score − 0.20 (±0.8). There were no differences between prescan vs postscan fullness ratings ($P > 0.10$). Both mean liking ($t_{(35)} = 6.65$, $P < 0.001$) and wanting ($t_{(35)} = 5.80$, $P < 0.001$) ratings were higher for High ED compared with Low ED foods (Table 1). There were no differences in children's rated liking ($P = 0.56$) or wanting ($P = 0.42$) for all Large PS compared with Small PS food images.

Table 3. Region of interest results for main effects of portion size and energy density

Comparison	*Region of interest*		x	y	z	*F*	P
Large PS vs Small PS	Inferior frontal gyrus (IFG)	R	50	4	16	5.32	0.03[a]
High ED vs Low ED	Hypothalamus	L	− 3	− 1	− 4	5.43	0.02[a]

Abbreviations: ED, energy density; L, left hemisphere; PS, portion size; R, right hemisphere. [a]Survived FDR correction $P < 0.05$.

fMRI data

ROI analyses related to PS

Large PS vs Small PS: Figure 2 shows the mean BOLD magnitude in ROIs with significant main effects of PS. Greater activation was found in areas known to be involved in inhibitory control, the right and left IFG ($x, y, z = \pm 50, 4, 16$) in response to Large PS compared with Small PS food images (right IFG, $F_{(1,35)} = 5.3$, $P = 0.03$; left IFG $F_{(1, 35)} = 4.8$, $P = 0.04$). Regions that function in motivational drive and emotion (that is, limbic system) did not reach significance (Supplementary Table S3), including the right orbitofrontal cortex, left striatum and right parahippocampal gyrus (*P*-values ranging from 0.06 to 0.08). Only the effect in the right IFG in response to large vs small portions, irrespective of ED, remained significant after correction.[51] Upon adjusting for prescan fullness and liking, the main effect for PS in the right IFG was no longer significant ($P = 0.91$). There was no significant interactions between PS and ED in the right IFG ($F_{(1, 35)} = 0.04$, $P = 0.83$).

Large PS vs Scrambled: Increased activation was found in the left IFG ($t_{(35)} = 2.7$, $P = 0.01$, survived FDR correction) in response to Large PS compared with Scrambled images (Supplementary Table S2).

Small PS vs Scrambled: Reduced activation was found in the right orbitofrontal cortex ($t_{(35)} = -2.2$, $P = 0.04$, survived FDR correction) in response to Small PS compared with Scrambled images (Supplementary Table S2).

ROI analyses related to ED

High ED vs Low ED: Figure 2 depicts the main effect for ED ($F_{(1, 35)} = 5.4$, $P < 0.05$) found in the left hypothalamus ($x, y, z = -3, -1, -4$), which showed reduced activation to High ED vs Low ED foods. There was a trend for greater activation in response to High ED vs Low ED foods in two other reward-based regions, the right ventral tegmental area (VTA) ($F_{(1, 35)} = 3.4$, $P = 0.07$) and the red nucleus ($F_{(1, 35)} = 3.7$, $P = 0.06$) (Supplementary Table S3). Only activation in the left hypothalamus surpassed correction for multiple testing. After adjusting for fullness, the main effect of ED in the left hypothalamus remained significant ($F_{(1, 35)} = 4.2$, $P < 0.05$) but was no longer significant after adjusting for both fullness and liking of food images ($P = 0.58$). In the left hypothalamus, no significant interaction between ED and PS was observed ($F_{(1, 35)} = 0.2$, $P = 0.65$).

High ED vs Scrambled: Increased activation was found in the left dorsolateral prefrontal cortex ($t_{(35)} = 2.5$, $P = 0.02$, survived FDR correction) in response to High ED food images compared with Scrambled images (Supplementary Table S2).

Low ED vs Scrambled: Reduced activation was found in the right parahippocampal gyrus ($t_{(35)} = -2.2$, $P = 0.03$, survived FDR correction) in response to Small PS compared with Scrambled images (Supplementary Table S2).

DISCUSSION

The aim of this study was to determine how the children's brains respond to food images varied by PS and ED in predefined brain regions. Related to this objective, we found that images of large food portions activated the right IFG to a greater extent than images of small food portions. We also found that high relative to low energy food images were associated with reduced activation in the left hypothalamus. Our hypotheses were partially supported; brain areas involved with cognitive control were responsive to the amount of food presented and those involved with sensory/reward processing responded to food ED. These results extend the pediatric neuroimaging literature by providing evidence that food PS and ED may be processed in the brain

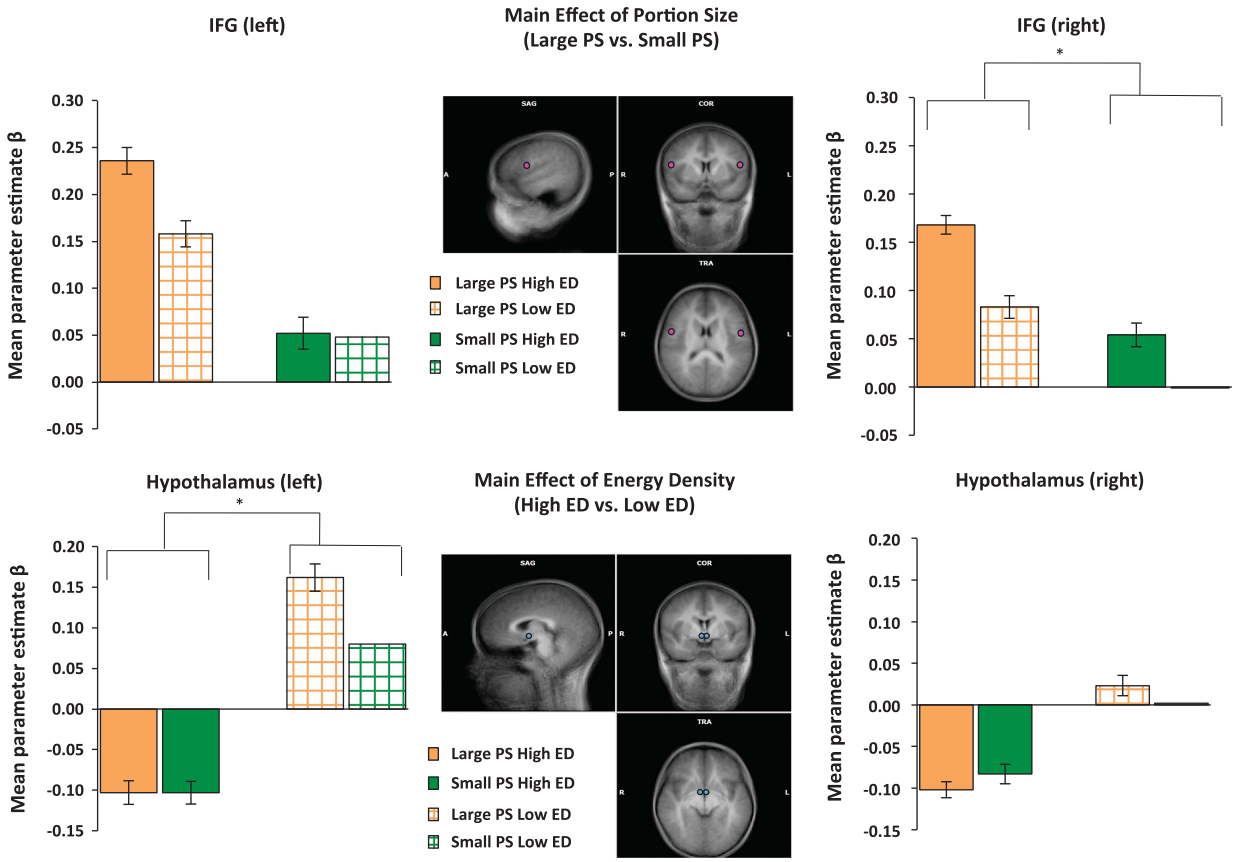

Figure 2. Graphs represent the main effects of PS (upper) and ED (lower) within ROI (center). Upper: In the bilateral IFG, the brain response to Large PS vs Small PS was elevated across all participants. Only the right IFG remained significant after correction (*$P < 0.05$). Lower: In the left hypothalamus, all children had reduced brain response for High ED compared with Low ED foods. Only the left hypothalamus was significant after correction (*$P < 0.05$). The right hypothalamus ($P = 0.10$) is shown for symmetry but did not reach significance before or after correction. Error bars denote the s.e. for each condition. Center: Colored spheres represent the ROI from which average parameter estimates (β) for mean BOLD signal were extracted. The ROIs were defined by drawing a 5-mm sphere at *a priori* coordinates in BrainVoyager QX (v2.8). All voxels $P < 0.05$, corrected.

differently from one another. When reproduced, these findings have implications for the development of more effective cognitive strategies to help children control intake from large portions of energy-dense foods.

One of our primary analyses showed that the IFG, a brain region involved in inhibitory processing,[53] was activated in response to food PS. Although only the right IFG survived correction, the effect sizes in both the left and right hemispheres were of similar magnitude. Previous studies have found greater bilateral IFG activation during food-specific no-go vs go trials[27] in response to unhealthy vs healthy food labels[54] and when making health-based vs value-based food choices.[43] Although no previous fMRI studies have explored the response to food PS, a recent study in adults[55] used electroencephalography to identify the middle frontal gyrus, part of the frontal inhibitory network,[53] as one of several brain regions that respond to pictures of meals systematically varied in PS. Because participants in that study were judging their ideal PS of multi-item meals, it is possible that other inhibitory regions are recruited when making complex PS judgments and only the right IFG is engaged when single foods are presented in less variety as we found in the present study. Taken together, these studies suggest that these frontal inhibitory regions may be involved in processing food PS information.

Previous neuroimaging studies have found greater activation in the IFG in response to food cues. In adults who were fasted for 2 h, Schur *et al.*[26] found greater activation in the right IFG in response to fattening vs non-fattening food images. Although children were in a fed state, Bruce *et al.*[32] found greater activation in the right IFG when comparing food vs non-food images. We speculate that the IFG response to large PS may reflect, more generally, an attempt to control an anticipated reaction to large food portions or trait-like responding. For example, if a parent tends to tell their child they cannot have 'too much', the child may anticipate needing to inhibit their responses to large PS. Alternatively, the IFG response to large PS food cues may be involved in state-dependent engagement of self-control relative to fullness level, for example, planning how much one anticipates consuming prior to a meal based on learned expectations of the satiation the food will provide.[37] These findings could differ in obese children, and future studies should focus on broadening the characteristics of the children studied.

Activation in the right IFG was no longer significant after inclusion of prescan fullness level or liking of food stimuli. Therefore, both liking and fullness level may have driven the response to food PS in the right IFG. Youth in this study were tested after a 2-h fast to achieve a neutral appetitive state and completed liking ratings after the scan. Because we did not find that food PS influenced liking ratings, we speculate that children rated liking based on the food pictured and not necessarily based on the amount of food pictured. Both children's liking of large portions and prescan fullness explained a significant amount of variance in our main findings in the right IFG. Consistent evidence showing that brain regions respond differently to food cues based on palatability[16,29] and level of fullness[22,23,30] support these

findings. Children who liked large portions of food, but were not hungry according to rated fullness, may have elicited a stronger inhibitory response when viewing large portions in the scanner. It should be noted that differences in low-level visual features (that is, edges[41]) between the large and small PS conditions may have contributed to the reported effect, although brightness between the conditions was not different. Follow-up studies that match conditions for liking and better control for prescan fullness levels as well as visual features of stimuli may be needed to disentangle these effects. This is one of the first studies in children to test differences in brain response to visual food cues that varied at a specific ED cutoff. Lower activation was found in the left medial hypothalamus in response to High ED vs Low ED food images, which remained significant following adjustment for rated fullness. Lower ED foods are presumably perceived as healthier, provide greater expected satiation[56] and may evoke greater responding in this region with known involvement in appetite regulation. The hypothalamus has a key role in the homeostatic control of appetite, and its connections to the VTA provide a critical pathway of communication between the homeostatic and hedonic, or reward-based, systems of feeding.[46] In overfed states, the left medial hypothalamus has shown reduced activation to hedonic relative to neutral food images in lean adults.[57,58] As part of the neural network involved in appetite regulation, the hypothalamus sends input to the VTA innervating other brain regions through the mesocorticolimbic dopamine pathway.[45] Although not significant, we found a trend for activation in the VTA for the ED comparison. In the opposite direction of our results, Schur *et al.*[26] found that, after a 2–4-h fast, healthy-weight women had increased activation in the right hypothalamus and midbrain to food images rated as fattening vs non-fattening. Additional studies are needed to determine whether similar findings would be seen in obese children or those tested in a different appetitive state.

The effect in the left hypothalamus described above was no longer significant after adjusting for liking of High ED foods. Previous fMRI studies in youth[17,18] and adults[25,30,31] report greater activation in brain regions with reward-based functions to higher energy food cues. Interestingly, a recent fMRI study that matched food stimuli for liking reported few differences in how the brain responds to foods of different energy contents presented at similar levels of palatability.[52] Therefore, it will be important for future fMRI investigations of food-cue responsiveness in children to match food stimuli for liking to separate its effects from food properties, such as PS and ED.

Several strengths and limitations should be acknowledged. This study helps to fill a gap in fMRI work with youth by testing an undersampled age group of children ranging in age from 7 to 10 years. However, this sample was homogenous in ethnicity and body weight, which reduces the generalizability of our findings. This study had a high success rate for scanning, which we attribute to extensive mock training. Although our testing protocol standardized the temporal spacing between events, all participants could not be tested at the same time of day owing to issues common to testing children such as school and extra-curricular activity schedules. Therefore, we cannot rule out the influence of time of day on these results, as adults have shown reduced activation to food images in the evening relative to daytime.[59] By converting children's anatomical data to Talairach space, we were able to use coordinates from regions reported in adult studies.[50] However, we acknowledge that no current spatial normalization process perfectly matches brains across individuals.[49] In addition, the food images used in the paradigm were not matched for palatability, although we adjusted for liking in our analyses. Our stimuli were balanced for brightness and integrated density, but it is possible that other low-level visual characteristics such as complexity and edges may have confounded the results. Furthermore, we cannot determine the extent to which brain responses to these images were due to differences in ED or the absolute caloric value of the foods. Although not always possible, we attempted to maximize the ED difference between two variants of a given food. Some ED values for the foods photographed were closer to the cut point than ideal (pork tenderloin, ED = 1.47 kcal g^{-1} and macaroni and cheese, ED = 1.54 kcal g^{-1}). However, because contrast values in fMRI are computed by averaging BOLD signal across a condition, the average ED within conditions is a more meaningful comparison. Mean ED for high and low ED conditions was 3.4 and 0.9 kcal g^{-1}, respectively. We did not include a control for object size (that is, large vs small furniture pieces) and acknowledge that brain regions responsive to different sized food portions could also be responsive to different sized non-food objects. Finally, preliminary test–retest data ($n = 5$) for Large PS vs Small PS in the right IFG demonstrated an intraclass correlation of 0.72, indicating good reliability of our paradigm, but further analyses are needed.

In conclusion, our hypotheses were partially confirmed by ROI-based analyses that revealed increased right IFG (for example, inhibitory control) activation to large relative to small portions and reduced left hypothalamus (for example, energy balance) activation to high relative to low ED food images. Overall, ROI analyses revealed distinct activation to PS compared with ED conditions of food stimuli in preadolescent children. Our findings indicate that children may be differentially responsive to PS and ED, food cues that are associated with energy intake. This study fills a gap in the neuroimaging literature and the results may aid future interventions aimed at reducing energy intake from large portions of palatable energy-dense foods.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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